

**CLINICAL PROFILE OF ULCERATIVE COLITIS
WITH SPECIAL REFERENCE TO RISK FACTORS AND
ESTIMATION OF COPY NUMBERS POLYMORPHISM OF
HUMAN BETA-DEFENSIN 2 GENE (DEFB4)**

**A dissertation submitted in part fulfillment of the requirements for DM
(Branch IV, Gastroenterology) examination of the Tamil Nadu Dr.
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CERTIFICATE

This is to certify that this dissertation entitled “**Clinical profile of ulcerative colitis with special reference to risk factors and estimation of copy number polymorphisms of defensin gene (DEFB4)**” is the bonafide work done by Dr. E. Umakanth in partial fulfillment of rules and regulations for DM (Branch IV, Gastroenterology) examinations of the Tamil Nadu Dr. M.G.R. Medical University, Chennai to be held in July 2008.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disorder that affects the colonic mucosa causing ulceration and leading to chronic blood and mucus diarrhoea. Although generally believed to be an immunological disorder, several decades of research have not unearthed the cause of UC. In the last seven years, genetically determined disorders of innate immunity have been identified as the cause of Crohn's disease, the other major inflammatory bowel disorder. UC is different from Crohn's disease in affecting only the mucosa and that too only in the large intestine. It is not clear whether it occurs in reaction to bacteria that exist within the lumen of the bowel. The large intestine is host to trillions of bacteria, and it is likely that the colonic mucosa has mechanisms to keep these microorganisms at bay and to maintain integrity of the mucosal barrier. It is conceivable that UC results from the breakdown of these mechanisms.

The innate immune system of the gut perceives pathogenic microorganisms through conserved molecular patterns (pathogen associated molecular patterns). Various effector molecules are synthesized and released in response to recognition of these pathogen associated patterns. The defensins are effector molecules of the innate immune system which function as anti-microbial peptides against pathogenic microbes. Reduced production of alpha-defensin by Paneth cells in the ileum is noted in patients with Crohn's disease. On the other hand, there are reports that mRNA for beta-defensins is up regulated in epithelial cells and colonic mucosal plasma cells in patients with UC, and that such levels return to normal with therapy. While upregulation of beta defensin mRNA may potentially be secondary to increased exposure to luminal bacteria, it is also

known that there may be variations in gene number for beta-defensins. This is a phenomenon known as copy number polymorphism. Defensin expression may be related to variation in the copy numbers of the defensin gene, since such variation is known to occur in man. This study was undertaken to determine whether patients with ulcerative colitis have variations in human beta-defensin gene copy numbers in comparison to control volunteers.

REVIEW OF THE LITERATURE

Ulcerative colitis (colitis ulcerosa, UC) is a chronic inflammatory bowel disorder (IBD) that affects the colon inducing characteristic ulcers. It affects the rectum and extends proximally along a variable length of colon. The main symptom of active disease is usually gradual onset of diarrhea mixed with blood. UC also affects many parts of the body outside the intestine. It is an intermittent disease with periods of exacerbation and periods of disease remission. Although the symptoms of ulcerative colitis can sometimes diminish spontaneously, the disease usually requires treatment to go into remission. It can usually be well controlled with medications. Most people live normal and productive lives. Control of the disease includes long term medical treatment and regular monitoring of complications.

History

1859: Dr. Samuel Wilks first described this disease as “Idiopathic Colitis”. He recognized disease distinct from bacillary dysentery. He reported the pathologic finding of dilated and thinned colon, and noted severe universal inflammation in a patient with this condition.

1905: Lockhart–Mummery, who invented the Electric sigmoidoscope, named features as “sigmoiditis”.

1909: Sir Arthur Hurst gave more complete description of ulcerative colitis. He described sigmoidoscopic appearances and differentiation from bacillary dysentery.

1913: Brown proposed ileostomy to rest the bowel with fecal diversion and allow it to heal.

1926: Geoffrey Bourney first reported ulcerative colitis in children

1940: Sulphasalazine was introduced as an effective drug against ulcerative colitis

1945: Dennis in Minneapolis recommended colectomy at the same time as ileostomy

1964: Dick et al conducted controlled trial and proved the efficacy of sulphasalazine

1964: Ehrenpreis and Ericson advocated colectomy early in the course of the disease

1950: Bryan Brooke Introduced end ileostomy

1950: Truelove and Witts proved steroids as effective medical treatment of UC

Epidemiology

The incidence and prevalence of UC varies with geographic location and ethnicity. With available epidemiological data; the areas with highest rates of reported incidence and prevalence of ulcerative colitis include North America, England, North Europe and Australia. In North America the incidence rates range from 6.0 to 14.3 cases per 100,000 person years and the prevalence ranges from 37-246 cases per 100,000 persons. In Europe ¹² the incidence rates range from 1.5 to 20.3 cases per 100,000 person years and the prevalence ranges from 21-243 cases per 100,000 person years.

There is a marked ethnic variation in the incidence of ulcerative colitis. One ethnic group with high incidence of this disease is the Jewish population. Incidence of ulcerative colitis in Jews is several folds higher than in the non-Jewish population. In United States it is 13 cases /100,000 in Jews compared to 3.8/100,000 in non Jews ⁽¹³⁾.Ulcerative colitis was traditionally considered uncommon in blacks. However studies in the late 1970s revealed incidence rates were comparable between whites and nonwhites in US. An increased incidence has been observed among blacks in South Africa, but still remains lower than that for South African whites ⁽¹⁴⁾.Ulcerative colitis in Asia is less common than in Western countries. The prevalence and annual incidence

rates for ulcerative colitis in Japan have remained relatively stable at 5.5 cases /100,000 and 0.36 to 0.5 cases/10,000 person respectively.⁽¹⁵⁾ The prevalence of ulcerative colitis in Indian has been reported to be substantially lower than among Europeans⁽¹⁶⁾ However, South Asian immigrants in England are more likely to have ulcerative colitis than European natives⁽¹⁷⁾. This changing epidemiology with immigrant population from low risk to high risk geographic region supports the concept of environmental influence on disease development⁽¹⁸⁾.

Ulcerative colitis can present at all ages although diagnosis below 5 years and above 75 years is uncommon, The peak incidence of ulcerative colitis occurs in the 2nd and 3rd decades of life and 2nd peak between 60—70 years. There is no gender difference at all ages⁽²¹⁾. Ulcerative colitis is more common in industrialized than in underdeveloped countries, among urban than rural population, higher socio economic stratum than low. These observations support the notion that environmental factors influence the development of ulcerative colitis.

Temporal influences:

There is a progressive increase in rates of ulcerative colitis incidence in last four decades, which was reported in studies in Europe⁽¹⁹⁾ and Sweden⁽²⁰⁾. It has been suggested that this trend reflects a parallel temporal change in some life style (environmental factors with increased exposure to an unidentified disease triggers (or) because of increased access to diagnostic technologies like fiberoptic endoscopy.

Environmental Risk Factors:

Only a few environmental exposures are widely accepted as altering an individual's risk of developing ulcerative colitis, as described below:

Smoking:

It is the best and most consistently documented environmental factor associated with ulcerative colitis and has a protective effect on the development of the disease.

Harries et al⁽²²⁾ in 1982 reported the lower than expected prevalence of smoking among patients with ulcerative colitis. Harries et al did a case control study⁽²³⁾ comparing the smoking habits of patients with ulcerative colitis and healthy control groups. They found

1. Current smokers are under represented among those with ulcerative colitis
2. Much of this under representation is due to a higher proportion of never smokers
3. Patients with ulcerative colitis who had smoked usually had quit before the onset of their disease.

Calkin⁽²⁴⁾ by metaanalysis found that life time non smokers were 2.9 times (95% CI, 2.6-3.2) more likely to develop ulcerative colitis than current smokers. Former smoker were also demonstrated to be at a higher risk compared with life time non-smokers (OR 1.7). They also found

- 1) A dose response association with higher levels of smoking being associated with a lower risk of disease and
- 2) A clustering of disease onset shortly after smoking cessation. Boyko et al⁽²⁵⁾ found current smokers had lower hospitalization rates than non smokers. Smoking has been shown to effect the immune system and inflammatory cascades, colonic mucus, intestinal permeability and circulating plasma cortisol concentration⁽²⁶⁾.

Oral Contraceptives:

Godet et al⁽²⁷⁾ did a meta analysis which showed oral contraceptive use was associated with a risk of 1.29 (95% CI, 0.94-1.77), but the authors did not find any

evidence of dose response relationship. A population based case control study in Washington state by Boyko et al ⁽²⁸⁾ found a relative risk of 2 (95%CI, 1.2 – 3.3). In ulcerative colitis there was no difference in risk with oral contraceptive use but a difference was seen based on estrogen dose. A cohort study⁽²⁹⁾ found that oral contraceptive use was an independent risk factor for recurrence following medical (or) surgical induction of remission, with a risk of 3 (95% CI, 1.5-5.9). A clear pathogenic mechanism for contraceptives in the development of IBD has not been defined.

Events in childhood: Infection, hygiene, and breast feeding:

Epidemiological studies have suggested a role for perinatal or early childhood events in the etiology of IBD. Early life events could also provide an explanation for geographic differences in disease incidence. Specific and non specific exposures have been studied to explain these epidemiological observations. Nonspecific exposures include early weaning, hygiene, gastroenteritis and other nonspecific infection. Specific exposures include measles, vaccines, appendicectomy, passive smoking and dairy products. Studying the role of early childhood events is clearly challenging especially since measuring exposure status usually relies on a subjects recall of early life events.

Acheson and Truelove ⁽³⁰⁾ in 1961 reported patients with ulcerative colitis likely to have been weaned early compared with healthy control. In 1979, Whorwell et al confirmed a higher prevalence of early weaning in subjects with ulcerative colitis.

Rigars et al found a relative risk comparing those who were breastfeed for less than 5 months to those who were never breast feed was 0.7 for both ulcerative and CD ⁽³²⁾. Breast feeding is known to provide immunological protection to the newborn and is important in the development of the intestinal mucosal barrier function.

Appendectomy:

Gilat et al ⁽³³⁾ noted a lower than expected rate of appendectomy in patients diagnosed with ulcerative colitis in childhood. Rutgerts ⁽³⁴⁾ reported that only one of 174 ulcerative colitis patients had undergone an appendectomy before the onset of their ulcerative colitis, a rate much lower than that seen in controls. A meta-analysis by Koutrarbakin et al ⁽³⁵⁾ found that appendectomy reduced the risk of ulcerative colitis by 69% (95% CI 62% - 75%). Anderson et al ⁽³⁶⁾ performed a large cohort study using Swedish population-based health registries and found negative association between appendectomy and the subsequent development of ulcerative colitis but only for those who underwent appendectomy for inflammatory conditions (appendicitis and mesenteric lymphadenitis) but no protective effect in those who underwent appendectomy for nonspecific abdominal pain and also found to be useful only if performed before age 20 years. Anderson et al argue that their findings suggest that it is not the appendectomy that is protective but the inflammatory process that led to the appendectomy possibly reflecting a predisposition to a type-1 helper T-cell inflammatory response rather than the type 2 response.

Etiology And Pathogenesis:

The etiology of ulcerative colitis is presently unknown but is likely multifactorial. It involves complete interaction of three elements:

1. Genetic susceptibility
2. Host immunity and
3. Environmental factors.

Dysregulation of the enteric immune response in genetically predisposed individuals leads to the development of acute and chronic inflammation and the pathological features of mucosal damage. The specific inciting antigens for the inflammatory process have yet to be identified, but several sources have been suggested including pathogenic and commensal micro organisms, metabolic by-products of these agents and normal epithelial structures.

Genetics:

Genetic factors have been limited to the development of ulcerative colitis. Family history is one of the most important risk factors for development of the disease. Kirsner & Spencer in 1963 first observed familial occurrence of disease ⁽³⁹⁾. 10-20% of the patients have at least one other affected family member. This familial association occurs in first degree relatives. North American and European studies have found a relative risk of 7-17 in the siblings of an individual with ulcerative colitis. Parent's off-springs and second degree relatives appear to be at lower risk for development of ulcerative colitis than all the first degree relatives. Parent sibling concordance is more common in United States but disease concordance is mostly between siblings in United Kingdom.

Jewish population which has a higher incidence of IBD has greater familial association. The life time risk of development is three fold higher among first degree relations of Jewish patients ⁽³⁷⁾. A similar increase in risk also has been observed in relatives of patients with early onset of disease. Clinical characteristics of familial disease also have shown that onset of disease in a child is noted at a much earlier age than in the affected parent, but there is a high degree of concordance between affected siblings for age of onset.

For all affected first degree relatives with in a family there is a high concordance for type of disease (ulcerative colitis vs Crohn's disease), extent of disease and occurrence of extra intestinal manifestations. Twin studies have given strongest evidence of genetic influence for ulcerative colitis. Large European twin pair studies showed approximately 6-10% of monozygotic twin pairs had concordant ulcerative colitis compared with 0 to 5% of diagnostic twin pairs ⁽⁴⁰⁻⁴²⁾. Linkage studies have suggested that there were susceptibility genes for ulcerative colitis at chromosomes 2, 3, 6, 7 and 12 ^(43, 44). IBD 2 Locus on chromatic 12 appears to have the strongest linkage ⁽⁴⁵⁾ demonstrated in studies involving large number of families with ulcerative colitis ⁽⁴⁵⁾.

The MDRI gene product P.glycoprotein is highly expressed in intestinal epithelial cells and serves an important barrier function against xenobiotics. Animals deficient for the MDRIa gene develop a spontaneous colitis resembling ulcerative colitis ⁽⁴⁶⁾. There are also genes that appear to influence disease behavior independently of susceptibility genes. The following positive HLA associations have been found in various studies.

- Ulcerative colitis in the Japanese population and HLA-B5 (or the HLA-B5 subtype, HLA-B52 ⁽⁴⁷⁾.
- Ulcerative colitis in the Israel: Jewish population and HLA-35 ⁽⁴⁸⁾
- Ulcerative colitis and HLA –DR2 (particularly the molecularly – determined DRB1* 1502 allele in Japanese and Jewish populations ⁽⁴⁹⁾

There also are genes that appear to influence disease behavior independently of susceptibility genes. The best studied of these are the human leukocyte antigen (HLA) alleles. One allele of HLA-DR2 (*DRB1*1502*) appears to be involved in disease susceptibility in Japanese and Jewish populations. Several centers have reported an

association between severe disease and a rare allele of HLA-DR1 (*DRB1*0103*). In some studies, the HLA-DR3, DQ2 haplotype is associated with extensive colitis, especially among women. Among the Jewish population, the peri-nuclear antineutrophil antibody (pANCA) is a marker for the *DRB*1502* allele of HLA-DR2, but in non-Jewish whites, this antibody is associated with the HLA-DR3 DQ2-tumor necrosis factor (TNF)- α_2 haplotype.

Immunopathogenesis:

The prevailing theory of the pathogenesis of UC emphasizes the role of the enteric immune response. The physiologic state of the intestine is one of constant low-grade inflammation in response to environmental stimuli such as bacterial products or endogenous factors. Breaches in this well-regulated mucosal immune system lead to the chronic uncontrolled mucosal inflammation observed in UC. In this regard, immunologic mechanisms in the pathogenesis of UC involve both humoral and cell-mediated responses.

Humoral Immunity

Histological examination of the inflamed colon indicates a marked increase in the number of plasma cells. This increase is not uniform among cells producing different classes of immunoglobulins. The largest proportional increase occurs in IgG synthesis, which has the highest pathogenic potential among antibody classes. The increase in IgG synthesis in UC is most pronounced in the IgG₁ and IgG₃ subclasses^(51, 52).

The increased IgG synthesis in ulcerative colitis may represent polyclonal stimulation; however, patients with UC frequently have circulating antibodies to dietary, bacterial, and self-antigens that are mostly of the IgG isotope, usually the IgG₁ subclass.

Many of these antibodies are thought to be epiphenomena because the serum antibody titers do not correlate with clinical parameters. Nevertheless, the known cross-reaction between enterobacterial antigens and colonic epithelial epitopes may be an important triggering event, even though, later in the course of the disease, the serum antibody titer to either the bacterial or the colonic antigen may be unimportant.

UC is an autoimmune disease. This belief is supported by its increased association with other autoimmune disorders, including thyroid disease, diabetes, and pernicious anemia⁽⁵³⁾. Patients with UC have varying levels of autoantibodies to lymphocytes, ribonucleic acid, smooth muscle, gastric parietal cell, and thyroid antibodies; these are neither tissue nor disease specific. The best characterized intestinal auto antigen is an epithelial antigen of 40-kd size found in normal colonic epithelium⁽⁵³⁾. This auto antigen is recognized by IgG eluted from the inflamed colonic mucosa of patients with UC and is a component of the tropomyosin family of cytoskeleton proteins⁽⁵⁴⁾. This autoantibody has the potential to activate complement *in vivo*, but direct evidence of antibody-induced cytotoxicity has not been observed. The antibody response to this 40-kd protein appears to be unique to UC.

pANCA is present in 60% to 85% of patients with UC^(55,56). It is synthesized within the lamina propria and is of the IgG₁ subclass. The antigen to which the pANCA is directed has not yet been determined with certainty. Recent evidence suggests that the antigen is a 50-kd nuclear envelope protein that is specific to myeloid cells⁽⁵⁷⁾. The pathogenic relevance of pANCA in UC is unknown. It may serve as a potential marker of susceptibility and genetically distinct subsets of UC. pANCA may be associated with a

more aggressive disease course⁽⁵⁸⁾ and the development of pouchitis following ileal pouch-anal anastomosis (IPAA) in patients with UC⁽⁵⁹⁾.

Cellular Immunity

Immune dysregulation in UC also involves cell-mediated immunity. In patients with UC, the absolute number of IELs is normal or reduced. Most of these cells are CD8⁺ cells, and the function of IELs has not been well characterized. It has been suggested that they are cytotoxicity and also may be active in suppressing local immune response⁽⁶⁰⁾. In patients with UC, the proportion of IELs using the $\gamma\delta$ T cell receptor may increase. However, the function and significance of $\gamma\delta$ T cells are unknown. Studies have suggested that T cell receptor repertoire is altered in active IBD⁽⁶¹⁾. Nonspecific cellular immunity also is altered. In patients with active disease, there is an overproduction of circulating monocytes as well as mucosal macrophages⁽⁶²⁾. The inflamed mucosa of patients with UC also exhibits infiltration of substantial numbers of granulocytes.

Epithelial Cells

Intestinal epithelial cells serve barrier functions and play a role in enteric immunity. Colonocytes express class II antigens and can function as antigen-presenting cells⁽⁶³⁾. In addition, they also express cytokine receptors, secrete various cytokines and chemokines, and express leukocyte adhesion molecules^(64, 65, and 66). Thus abnormalities in colonic epithelial cells can contribute to the development of UC. Patients with UC have an increased turnover rate of colonic epithelium⁽⁶⁷⁾ and other abnormalities of epithelial cells include a reduced metabolism of short-chain fatty acids, especially butyrate, abnormal membrane permeability,⁽⁶⁸⁾ and altered composition of glycoprotein mucus produced by the colonic epithelium⁽⁶⁹⁾.

Consequences of Immune Activation

Activation of macrophages, lymphocytes, and colonic epithelial cells leads to the release of a variety of cytokines and mediators that further amplify the immune and inflammatory response of UC and result in tissue damage. Based on the cytokines they produce, CD4⁺ T cells have been divided into two major immune phenotypes: T helper 1 (Th1) and T helper 2 (Th2). The Th2 response is characterized by the production of cytokines IL-4, IL-5, and IL-10, which amplify the humoral immune response. Both Th1 and Th2 pathways can be regulated by unique regulatory T cells (Th3, T regulatory 1) subsets that produce IL-10 and transforming growth factor- β and down-regulate inflammation ⁽⁷⁰⁾. Macrophages in the inflamed colon in patients with active UC synthesize IL-1 β , TNF, and IL-6, whereas lamina propria T cells probably produce IL-2 and IFN- γ . This immune response can be up-regulated further by presentation of antigen to CD4⁺ lymphocytes by colonic epithelial cells that express HLA class II antigens ⁽⁶³⁾. Release of these cytokines also may lead to other abnormalities seen in UC, such as increased epithelial cell permeability and collagen synthesis. Alteration of endothelium by a variety of cytokines may result in local ischemia. Increased expression of endothelial adhesion molecules in response to inflammatory mediators recruits circulating granulocytes and monocytes to the inflamed tissues, thus further perpetuating the inflammatory response. Mucosal concentrations of many mediators have been shown to be elevated in patients with active UC, including leukotrienes, thromboxane, platelet-activating factor, nitric oxide, and reactive oxygen metabolites ⁽⁷¹⁾. These mediators, which are mostly released from active macrophages and neutrophils, contribute to inflammation and mucosal injury, altered epithelial cell permeability, thereby further contributing to

diarrhea. Diarrhea in UC also is caused by complement activation and the release of kinins and other inflammatory mediators from mast cells and eosinophils.

Pathology

45% of patients with UC have disease limited to the recto sigmoid, 35% have disease extending beyond the sigmoid but not involving the entire colon, and 20% of patients have pan colitis⁽⁷²⁾. UC starts most severe distally and progressively less severe more proximally, continuous and symmetrical involvement is the hallmark of UC. 75% of patients with left-sided UC may have appendiceal inflammation and patchy inflammation in the cecum⁽⁷³⁾ resembling the skip pattern characteristic of Crohn's disease.

Macroscopically: the mucosa in UC appears hyperemic, edematous, and granular in mild disease. As disease progresses, the mucosa becomes hemorrhagic with visible punctate ulcers. These ulcers may enlarge and extend into the lamina propria. They often are irregular with overhanging edges or may be linear along the line of the teniae coli. Epithelial regeneration with recurrent attacks results in the formation of pseudopolyps; Long-standing disease can cause atrophic and featureless colonic mucosa, associated with shortening and narrowing of the colon.

Microscopically: the early stage of UC is marked by edema of the lamina propria and congestion of capillaries and venules, often with extravasations of red blood cells. This is followed by an acute inflammatory cell infiltrate of neutrophils, lymphocytes, plasma cells, and macrophages. There also may be increased numbers of eosinophils and mast cells. Neutrophilic infiltration of colonic crypts gives rise to cryptitis and ultimately to crypt abscess with Neutrophilic accumulations in the crypt lumen. This migration of

neutrophils from the circulation into the lamina propria occurs in response to a variety of chemo attractants, including chemo tactic peptides of colonic bacteria, IL-8, activated complement, platelet-activating factor, and leukotriene B₄. The cryptitis is associated with discharge of mucus from goblet cells and increased epithelial cell turnover.

Inflammation in UC characteristically is confined to the mucosa. The inflammatory changes typically end at the luminal aspect of the muscularis mucosa. With increasing inflammation, however, the surface epithelial cells become flattened and eventually ulcerate. Deep ulceration may undermine the surrounding epithelium.

During the healing phase of UC, the inflammatory infiltrate subsides and epithelial regeneration takes place. Epithelial cells undergoing regenerative changes become cuboidal with eccentric, large nuclei, and prominent nucleoli. These features may be confused with dysplasia.

A classic histological feature of chronic quiescent UC is crypt architectural distortion or actual dropout of glands. Architectural changes include branching or bifid glands, wide separation among glands, and shortened glands that do not extend down to the muscularis mucosa. Whereas architectural alteration is a prominent feature of chronic quiescent UC, the histologic abnormalities may revert to normal after mild flares in the early course of disease.

Most of these findings are not specific for UC. Histologic severity of inflammation does not necessarily correlate with clinical disease activity in patients with UC and patients may be relatively symptom free, while histology reveals significant inflammation.

Clinical Features

Patients with UC may present with a variety of symptoms. Bloody diarrhoea (96%) is the cardinal feature of acute ulcerativecolitis, which in most patients runs a clinical course characterized by unpredictable relapses interspersed with periods of remission. Other common symptoms include passage of mucus, tenesmus, urgency, and abdominal pain (mild to moderate in 55%)⁽⁷⁷⁾. In more severe cases, fever and weight loss (>5 kg in 12%)⁽⁷⁸⁾ may be prominent.

The symptom complex tends to differ according to the extent of disease⁽⁷⁴⁾. Patients with proctitis often have tenesmus, urgency, mucus, and bleeding, whereas patients with extensive colitis may have more diarrhea, weight loss, fever, clinically significant blood loss, and abdominal pain.

The onset of UC typically is slow and insidious. The median interval between the onset of symptoms and diagnosis of UC is approximately 9 months⁽⁷⁵⁾. A paper by Felicity Edwards and Truelove et al⁽⁷⁶⁾ observed the duration of symptoms before first presentation was <1month in 19.6 % , 1-6 months in 58.4% and 22% in 6-12months . Age of onset was <20 yrs in 10 % , 20 – 59yrs in 72.8% and >60yrs in 17.2%. The median decade of onset was 30 – 39 yrs (26.9%) and there was no second peak in older age, although 3.2% presented over the age of 80.

Symptoms

Rectal Bleeding

Rectal bleeding is common in UC. The characteristic of the bleeding is determined by the location of the disease. Patients with proctitis usually complain of

passing fresh blood, either separately from the stool or streaked on the surface of a normal or hard stool ⁽⁷⁹⁾. Patients with ulcerative proctitis often pass a mixture of blood and mucus and may even be incontinent. Patients with proctitis also often complain of the frequent and urgent need to defecate, only to pass small quantities of blood and mucus without fecal matter.

When the disease extends proximal to the rectum, blood usually is mixed with stool or there may be grossly bloody diarrhea. When the disease activity is severe, patients pass liquid stool containing blood, pus, and fecal matter.

Diarrhea

Diarrhea is common but not always present in patients with UC. Up to 30% of patients with proctitis or proctosigmoiditis may complain of constipation and hard stools ⁽⁷⁹⁾. Most patients with active disease complain of frequent passage of loose or liquid stools and may have nocturnal diarrhea. Fecal urgency, sensation of incomplete evacuation, and fecal incontinence also are common, especially when the rectum is severely inflamed. Diarrhea in this setting often is accompanied by passage of large quantities of mucus, blood, and pus.

The Pathophysiology of the diarrhea in UC involves several mechanisms, 1) failure to absorb salt and water is perhaps the predominant factor ⁽⁸⁰⁾, This failure results from reduced Na^+/K^+ , ATPase pump activity, increased mucosal permeability, and altered membrane phospholipids. Urgency and tenesmus, which are common symptoms when the rectum is inflamed, are caused by poor compliance and loss of the reservoir capacity of the inflamed rectum ⁽⁸¹⁾ with severe inflammation, the urgency can be sufficiently acute as to cause incontinence.

Colonic motility is altered by inflammation, and there is rapid transit through the inflamed colon. With left-sided disease, distal transit is rapid, but there is actual slowing of proximal transit,⁽⁸²⁾ which may help explain the constipation that is commonly seen in patients with distal colitis. Prolonged transit times in the small intestine also occur in the presence of active colonic inflammation⁽⁸²⁾.

Abdominal Pain

Many patients with UC complain of abdominal pain with active disease, but pain generally is not a prominent symptom. Patients may experience vague lower abdominal discomfort, an ache in the left iliac fossa, or intermittent abdominal cramping preceding bowel movements and often persisting transiently after defecation. Severe cramping and abdominal pain can occur in association with severe attacks of the disease.

Pathophysiology of pain is not clear, probably related to the increased tension within the inflamed colonic wall during muscular contraction. Patients with active proctitis also frequently complain of tenesmus and urgency associated with painful straining and passage of mucus and blood with only scanty stools.

Other Symptoms

Disease of moderate or severe activity often may be associated with systemic symptoms. Patients may develop anorexia and nausea. Protein loss through inflamed mucosa, hyper catabolism, and down-regulation of albumin synthesis caused by the inflammation, account for weight loss and hypoalbuminemia. Fever usually accompanies severe attacks. A few patients may present with extra intestinal manifestations, such as acute arthropathy, episcleritis, and erythema nodosum. These manifestations typically parallel the activity of colitis.

Signs

Patients with mild or even moderately severe disease exhibit few abnormal physical signs. They are usually well nourished and well appearing and show no signs of chronic disease. Indeed, these patients can appear deceptively well. Weight should always be recorded, and, for children and adolescents, both height and weight should be plotted on developmental growth charts. The affected portion of the colon may be tender on abdominal palpation, but this generally is mild and not associated with rebound or guarding. Bowel sounds are normal. Digital rectal examination also is frequently normal, but the rectal mucosa may feel “velvety” and edematous; the anal canal may be tender; and there may be blood on withdrawal of the examining finger.

Patients with severe attacks also may appear well, but most are ill with tachycardia, fever, orthostasis, and weight loss. The abdomen typically is soft with only mild tenderness over the diseased segment. Abdominal tenderness may become diffuse and moderate with more severe disease. Bowel sounds may be normal or hyperactive but diminish with progressive disease. In fulminant colitis, the abdomen often becomes distended and firm, with absent bowel sounds and signs of peritoneal inflammation. There may be aphthoid ulceration of the oral mucosa. Clubbing of the fingernails is a frequent manifestation of chronic disease. Peripheral edema may occur secondary to hypoalbuminemia. Minor perianal disease may be present but is never as severe as is seen in patients with Crohn's disease. Signs of extraintestinal manifestations also may be present.

Extra Intestinal Manifestations

These extra intestinal manifestations may affect virtually every organ system, but the most commonly involved organs are the skin, eyes, mouth, joints, and liver. These extra intestinal complications are often classified by their relations to the activity of the colitis, but they may occur before, during, or following exacerbations of bowel disease. Manifestations that parallel disease activity usually improve on treatment of the colitis.

Common Extra Intestinal Manifestations of Ulcerative Colitis

System	Manifestations
Musculoskeletal	Peripheral arthropathy
	Ankylosing spondylitis
	Sacroiliitis
	Osteopenia
	Osteoporosis
	Osteomalacia
	Osteonecrosis
Dermatologic	Erythema nodosum
	Pyoderma gangrenosum
	Oral ulcerations
	Angular stomatitis
	Aphthous stomatitis
	Pyostomatitis vegetans
	Psoriasis
Ophthalmologic	Sweet's syndrome (acute febrile neutrophilic dermatosis)
	Uveitis/iritis
	Episcleritis
	Scleritis
	Conjunctivitis
Hematologic	Retinal vascular disease
	Iron deficiency anemia
	Autoimmune hemolytic anemia

System	Manifestations
	Anemia of chronic disease
	Leukocytosis or thrombocytosis
	Leukopenia or thrombocytopenia
	Hypercoagulable state
	Coagulation abnormalities
Hepatobiliary	Steatosis
	Primary sclerosing cholangitis
	Pericholangitis
	Cholangiocarcinoma
	Autoimmune hepatitis

Laboratory Findings

Laboratory findings in UC are nonspecific and reflect the severity of the underlying disease. Patients with limited distal disease often pass visible blood in the stool, but the amount of blood loss typically is small and anemia, if present, is mild. Patients with active extensive disease or severe distal disease may have anemia, leukocytosis and thrombocytosis, which reflect active disease. In contrast, patients with quiescent UC typically manifest no laboratory abnormalities. Iron deficiency anemia may be present because of chronic blood loss. Anemia also may be present secondary to bone marrow suppression resulting from chronic inflammation or medications, such as azathioprine, 6-mercaptopurine (6-MP), and sulfasalazine.

Mild or moderate attacks rarely are associated with any biochemical disturbance. Hypokalemia, metabolic alkalosis, and elevated serum creatinine may be present in severe flares of UC, reflecting volume depletion. Hypoalbuminemia may be seen with both acute and chronic disease. Minor elevations in serum levels of aspartate

aminotransferase or alkaline phosphatase also are frequently associated with severe disease; these abnormalities probably reflect a combination of fatty liver, sepsis, and poor nutrition.

Serum inflammatory markers including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) may be elevated in active disease. Measuring them may be useful to assess disease activity in individual patients, particularly if these values are normal during periods of inactive disease.

Diagnosis

The diagnosis relies on a combination of compatible clinical features, endoscopic appearances, and histologic findings.

Endoscopy

The hallmark of UC is symmetrical and continuous inflammation that begins in the rectum and extends proximally without interruption for the entire extent of disease. The earliest endoscopic sign of UC is a decrease or loss of the normal vascular pattern, with erythema and edema of the mucosa. Distortion or loss of vascular markings may be the only endoscopic evidence of UC in patients with quiescent disease. As the disease progresses, the mucosa becomes granular and friable. With more severe inflammation, the mucosa may be covered by yellow-brown mucopurulent exudates associated with mucosal ulcerations.

In UC, mucosal ulcerations occur in areas of inflammation; vary in size from a few millimeters to several centimeters; and may be punctate, annular, linear, or serpiginous. Finally, severe UC is associated with mucosa that bleeds spontaneously, and

there may be extensive areas of denuded mucosa from severe mucosal ulcerations with diffuse colitis.

Pseudopolyps may be seen in patients with long standing UC which indicate in active disease and result from inflamed, regenerating epithelium that is interposed among ulcerations.

There is a loss of the normal colonic architecture with long-standing inflammation that is characterized by muscular hypertrophy, loss of the normal haustral fold pattern, decreased luminal diameter, and shortening of the colon; a resultant featureless appearance of the colon in chronic UC gives rise to the so-called lead pipe seen on barium enema examination.

Radiology

Plain abdominal radiograph: gives information about

- 1) Thickening of the colonic wall
- 2) Extent of the disease
- 3) Complications like toxic mega colon and perforation

Barium studies:

Endoscopy replaced barium studies in the care of patients with UC.

Barium studies of the colon still useful in

- 1) The evaluation of colonic strictures - location, length, and diameter and allows visualization of the entire colon when strictures preclude advancement of the colonoscope.
- 2) Upper gastrointestinal barium study and small bowel follow-through to exclude Crohn's disease.

- 3) Radiological changes in ulcerative colitis:
- 4) The earliest radiologic change of UC seen on barium studies is fine mucosal granularity
- 5) The mucosal line becomes irregular and becomes thickened and irregular, and superficial ulcers are seen
- 6) Deep ulceration can appear as “collar-stud” or “collar-button” ulcers in tangent, which indicates that the ulceration has extended through the mucosa to the muscularis propria
- 7) Loss of haustrations in patients with long-standing disease
- 8) Other chronic changes are shortening of the colon and widening of the presacral space as seen on a lateral film of the rectum.
- 9) Pseudopolyps may be present and often are filiform. In the presence of active changes, these pseudopolypoid changes can resemble a cobblestone pattern

Assessment of Disease Activity

A numerical disease activity instrument that is more useful for patients with limited disease. This index is the sum of scores from four components: stool frequency, rectal bleeding, sigmoidoscopic findings, and physician's global assessment. This disease activity index ranges from 0 to 12, with the higher total scores representing more severe disease. In general, a patient is considered to be in remission if the Ulcerative Colitis Disease Activity Index score is equal or less than 2 and to have severe disease if the score is greater than 10. Clinical response generally is accepted to be reflected by a decrease by 3 points from the patient's initial baseline score.

Ulcerative Colitis Disease Activity Index

Variable/Score	Criteria
Stool Frequency	
0	Normal
1	1-2 stools/day > normal
2	3-4 stools/day > normal
3	>4 stools/day > normal
Rectal Bleeding	
0	None
1	Streaks of blood
2	Obvious blood
3	Mostly blood
Mucosal Appearance	
0	Normal
1	Mild friability
2	Moderate friability
3	Exudation, spontaneous bleeding
Physician Global Assessment	
0	Normal
1	Mild
2	Moderate
3	Severe

* Range: 0–12.

SEO'S Activity Index:⁽⁸⁵⁾

Seo et al studied Quantification of disease severity in 72 patients with ulcerative colitis, who had undergone total 85 clinical courses. They performed a multiple stepwise regression analysis, according to Truelove and Witts' classification, with disease severity as a dependent variable, and with 18 clinical, laboratory, and endoscopic parameters as independent variables. Results showed that disease severity in patients with ulcerative

colitis is significantly influenced by five factors, namely, bloody stool, bowel movements, erythrocyte sedimentation rate (ESR), hemoglobin (Hb), and serum albumin. The activity index (AI) developed for ulcerative colitis is expressed as follows: $AI = 60 \times \text{blood stool} + 13 \times \text{bowel movements} + 0.5 \times \text{ESR} - 4 \times \text{HB} - 15 \times \text{albumin} + 200$. Index values below 150, values between 150 and 220, and values above 220 nearly corresponded to mild, moderate, and severe disease, respectively, in Truelove and Witts' classification.

MEDICAL THERAPY:

The goals of therapy of UC are

- (1) To induce remission
- (2) To maintain remission
- (3) To maintain adequate nutrition
- (4) To decrease disease- and treatment-related complications
- (5) To improve the quality of life. The current management strategy focuses on using appropriate medical therapy and optimizing timing of surgery.

Current therapeutic strategies can be classified broadly based on disease activity into those that treat active disease (induction therapy) and those that prevent recurrence of disease once remission is achieved (maintenance therapy)

The extent of disease in any given patient is an important consideration to help determine the route of administration of medication. Enema preparations may be used alone or in combination with systemic therapy for patients with left-sided disease or disease distal to splenic flexure. Proctitis or disease limited to the rectum may be treated with suppositories or foam preparations. Other important factors to consider are prior

response to or side effects from a specific medication and patient compliance. These factors might favor or preclude the use of a specific agent.

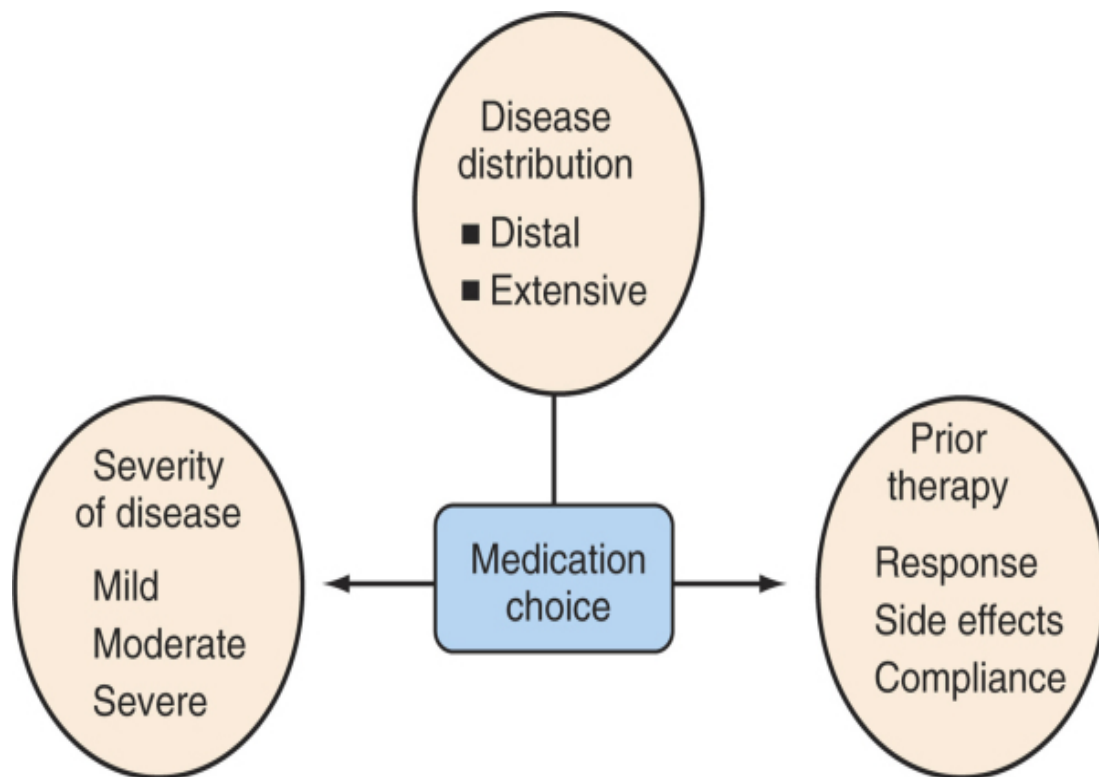


Table – 1. Induction Therapy for Ulcerative Colitis Depending on Disease Severity

Mild disease	Moderate disease	Severe disease
5-aminosalicylates	5-aminosalicylates	IV glucocorticoids
Topical (distal colitis)	Topical (distal colitis)	
Oral (distal/extensive colitis)	Oral (distal/extensive colitis)	IV cyclosporine
Combination	Combination	
	Glucocorticoids	IV infliximab
	Topical (distal)	
	Oral (distal/extensive)	
	Combination	
	Azathioprine or 6-mercaptopurine	

IV, intravenous.

Table -2 -- Maintenance Therapy for Ulcerative Colitis

Preparation	Type
5-Aminosalicylates	TopicalDistal
	Oral (distal/extensive)
Azathioprine or 6-mercaptopurine	Oral for severe diseases and steroid sparing.

DEFENSINS

Defensins are small cationic antimicrobial peptides that form an important part of the innate immune system and protect the intestinal mucosa against bacterial infection. 86 and play an important role in host immune system to some infectious diseases, immune diseases and skin disease Hexamers of defensins create voltage-dependent ion-channels in the target cell membrane causing permeabilization and ultimately, cell death

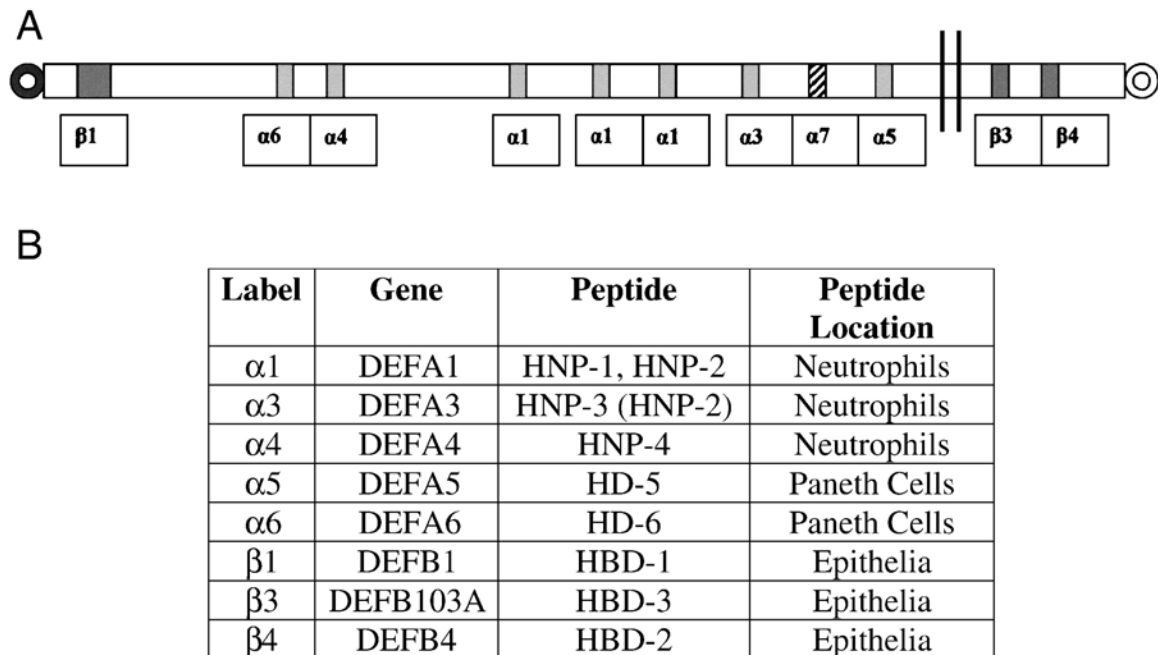
It has been suggested that deficient defensin expression may underlie the chronic inflammation of Crohn's disease. The DNA copy number of the β -defensin gene cluster on chromosome 8p23.1 is highly polymorphic within the healthy population, which suggests that the defective β -defensin induction in colonic CD could be due to low β -defensin-gene copy number ⁽⁸⁹⁾.

Defensins' are two types alpha(α) and Beta(β)-defensin. The α -defensins are the first group discovered and until now six human α -defensins were detected ⁽⁹⁰⁾. α -defensins are strongly expressed in neutrophils and are also present in certain epithelia, such as gut wall, endocervix and vagina. β -defensins are expressed in a variety of epithelia of the respiratory air ways ⁽⁹¹⁾. α -defensins are closely related to the β -defensins, differing in the spacing pattern of conserved cysteine residues and the linkage arrangements of the distal cysteine bands. These genes have been mapped to 8p22-p23 (Figure 1). Several types of beta-defensin are noted. DEFB4 is effective against *Escherichia coli* and prevention of *Pseudomonas aeruginosa* infection at micromolar concentration. The anti microbial properties of DEFB4 were expressed in leukocytes and acts as a chemokine for cells of the adaptive immune response ⁽¹⁰²⁾. Recent studies identified that the genes coding for human β -defensin 2 (HBD-2 or DEFB4), human β -

defensin 3 (DEFB103) and human β defensin 4 (DEFB104) showed variation in copy numbers. In Crohn's disease, reduced Paneth cell production of α -defensins in the ileum is considered to be a significant factor in pathogenesis of the disease. In ulcerative colitis, studies have shown that epithelial cells and plasma cells in the colon have increased expression of human β -defensin 2 (or DEFB4) mRNA. This increased expression of the defensins may be due to increased bacterial load in the lumen. Alternatively it may be genetically regulated, by the copy number polymorphisms of the DEFB4 gene. This variation may have an impact on gene expression levels. There has been no study of copy number polymorphisms of DEFB4 and ulcerative colitis, so the present studies were designed to measure the DEFB4 gene copy numbers in patients with ulcerative colitis⁽¹¹⁶

– 120)

Figure1. Defensin genes.



AIMS OF THE STUDY

To perform a case control study in patients with ulcerative colitis and matched controls:

1. To determine whether there are specific associations of ulcerative colitis with factors connected with childhood hygiene, and effect of appendectomy or tobacco usage on incidence of ulcerative colitis.
2. To determine the frequency of DEFB4 gene copy number polymorphisms in patients with ulcerative colitis and matched control volunteers. Normally each individual has two copies of a gene. However, certain genes are present in multiple repeats, and this number varies between individuals. This is known as a copy number polymorphism.

MATERIALS

SUBJECTS

CASES: Confirmed cases of ulcerative colitis diagnosed by clinical history, colonoscopic findings and histology. The clinical history consisted of history of blood and mucus diarrhoea presently or in the past. Colonoscopic findings of presence of symmetrical and continuous inflammation that began in the rectum and extended proximally without interruption with features of loss of normal vascular pattern, erythema and edema of mucosa with or without granularity, friability with presence of yellow brown mucopurulent exudates associated with mucosal ulceration which can be punctate, annular, linear or serpiginous and +/- presence of pseudopolyps. Histology showing cryptitis or crypt abscesses and absence of granulomas. Histologically the disease was graded as quiescent, mild, moderate and severe depending on the activity. Quiescent colitis was characterized by presence of architectural alterations with or without the presence fibrosis with no features of neutrophils infiltration, no edema no ulcers. Mild, moderate, severe disease were divided depending on the

- 1) Degree of neutrophil infiltration of the crypts and depth of mucosal infiltration
- 2) Amount of mucin depletion
- 3) Amount of edema and congestion with or without presence of the ulcers.

Grade increases if the above features increase in severity.

CONTROLS: Controls included patients with irritable bowel syndrome who were matched for age, sex and geographical region with no history of blood or mucus in stools and no family history of IBD.

METHODS

The study was designed as a prospective case control study. It was presented to the institutional review board and the protocol and the consent form were approved.

All participants were investigated only as clinically indicated. In the case of patients with ulcerative colitis, this included colonoscopy and biopsy and tests to establish the general severity of the disease and complications. Informed written consent was obtained from all participants or the guardian. A detailed questionnaire including risk factors of ulcerative colitis was read out to the patients and controls and their responses were recorded in the form. All the responses were later entered in to a computer spreadsheet for analysis using a statistical program.

Questions regarding risk factors concerned with hygiene were demarcated as during “childhood” and “current”. The reason was to determine whether specific associations existed with childhood hygiene or with current state of hygiene. These included (1) residence in urban area (or) village, (2) history of intestinal parasitic infestation, (3) drinking water source for the household and (4) availability of toilets. Questionnaires also included a detailed evaluation of socioeconomic status based on the modified Kuppuswamy scale, a past history of appendicectomy, and a history of consumption of tobacco in any form.

A 10ml sample of venous blood was drawn by me or by one of the laboratory co-investigators into Vacutainers. DNA was isolated from the whole blood and stored at -20°C for copy number polymorphism analysis.

DNA Extraction and Polymorphic Analysis:

DNA isolation from blood

Materials required:

1. EDTA coated vacutainer tube
2. Centrifuge
3. Plastic pasteur pipette (sterile)
4. 15 ml self standing centrifuge tube (sterile)
5. RBC lysis buffer
6. WBC lysis buffer
7. Proteinase K
8. 10% SDS
9. Saturated NaCl
10. Absolute alcohol
11. Eppendorf tubes (sterile)
12. 70% ethanol
13. TE buffer

Reagent preparation:

RBC lysis buffer:

Dissolve 8.275 g of ammonium chloride and 1 g of potassium bicarbonate in 1 liter of water. Check the pH and that should be 7.4. Sterilize the solution by autoclaving.

Can be stored at room temperature for a period of 1 year.

WBC lysis buffer:

Mix 25ml of 0.5 M EDTA and 2.19 g of NaCl in 250ml of water and adjust the pH to 8.0 and make up the volume to 500 ml with water. Sterilize by autoclaving. Can be stored at room temperature for 3 months.

Tris-EDTA buffer (10 mM Tris & 0.1 mM EDTA):

Mix 1 ml of 1 M Tris (pH 8.0) and 400 μ l of 0.25 M EDTA (pH 8.0) and make up the volume to 100 ml with water. Sterilize by autoclaving. Can be stored at room temperature for 1 year.

10% SDS:

Dissolve 10 g of SDS in 100 ml of water and heat the solution at 65° C to assist dissolution. Sterilization is not required. Can be stored at room temperature for 6 months.

Proteinase K:

Dissolve the lyophilized Proteinase K (Cat no.PK1, genei) in 10 ml of sterile water to obtain a concentration of 10mg/ml. Aliquot 500 μ l volume into Eppendorf tubes. Can be stored at –20° C for a long period.

Saturated NaCl:

Dissolve 36.05 g of NaCl in 100 ml of water and is saturated or 6M NaCl solution. Sterilize by autoclaving and store at room temperature for up to 1 year.

(Note: Always use milliQ water for the reagents preparation)

Procedure:

1. Collect the blood in EDTA-coated vacutainer tube.
2. Centrifuge the blood sample at 4000 rpm for 15 min.

3. Three layers will be visible: the top yellow layer is the plasma, the middle white layer is the buffy coat and the bottom red is the erythrocytes. Carefully remove the plasma and discard it and collect the buffy coat in a fresh tube.
4. Add ten volumes of RBC lysis buffer and incubate at room temperature for 10 min.
5. Then centrifuge the tube at 4000 rpm for 10 min. Discard the supernatant and a white pellet will be seen.
6. Repeat the wash until a clear white pellet is obtained.
7. Then to the pellet add 4.5 ml of WBC lysis buffer and make a homogenous solution. Add 250 μ l of 10% SDS and 100 μ l of Proteinase K and mix them well and incubate at 37° C for overnight or at 55° C for two hours.
8. Check the lysis, if the lysis is not complete, then add 50 μ l of Proteinase K and continue the digestion.
9. If the lysis is complete, add 1.5 ml of saturated NaCl solution and mix well till the solution turns milky white.
10. Centrifuge the solution at 4000 rpm for 15 min and collect the supernatant in a fresh tube.
11. To the supernatant add two volumes of absolute ethanol and mix gently, a white thread like structure will appear and will reach the top of the solution.
12. Collect the thread like structure in a fresh eppendorf tube containing 1ml of 70% ethanol, using a sterile pipet tip.
13. Tap the tube well to remove the excess salt adhering to the precipitate.
14. Remove the precipitate using a pipet tip and air-dry it.

15. When the precipitate turns transparent, transfer it into an eppendorf tube containing 200-300 µl of TE buffer.
16. The DNA can be dissolved by incubating at 37° C for 2 hr or 65° C for 30 min.
17. Store the DNA in deep freezer (-20 is adequate) for long-term storage or in the fridge for short term.

COPY NUMBER POLYMORPHISM ANALYSIS

DEBF4 copy number was assessed by quantitative real time polymerase chain reaction using SYBR Green method. PCR was carried out on a Chromo4 (MJ Research, USA) thermal cycler equipped with a continuous fluorescence detector and the PCR protocol was an initial denaturation step for 1 min at 95° C followed by 40 cycles consisting of denaturation step for 30 sec at 94° C, annealing step for 30 sec at 58° C, and extension step for 30 sec at 72° C and plate read to acquire the fluorescence and after 40 cycles a final extension step of 72° C for 5 min. Melting curve analysis was carried out to check the specificity and single peak was used to confirm the specificity. Copy numbers were calculated by normalizing against a gene with stable copy number. Here, Albumin was used as reference gene due to its stable copy numbers. Both DEFB4 and albumin were quantitated using specific primers forward: 5'- ctcacacagccctgaaaaca -3', reverse: 5'- accgtcactcaggggaatgtc -3' and forward: 5'- aggctcttttcttggaat -3', reverse: 5'- tgaacacccctccataagc -3' respectively, and the Ct values were used for copy number quantitation using the formula $2^{-\Delta Ct}$ ($\Delta Ct = DEFB4_{Ct} - Albumin_{Ct}$)⁽¹¹³⁾. The CNP was calculated and comparison was made between case and controls.

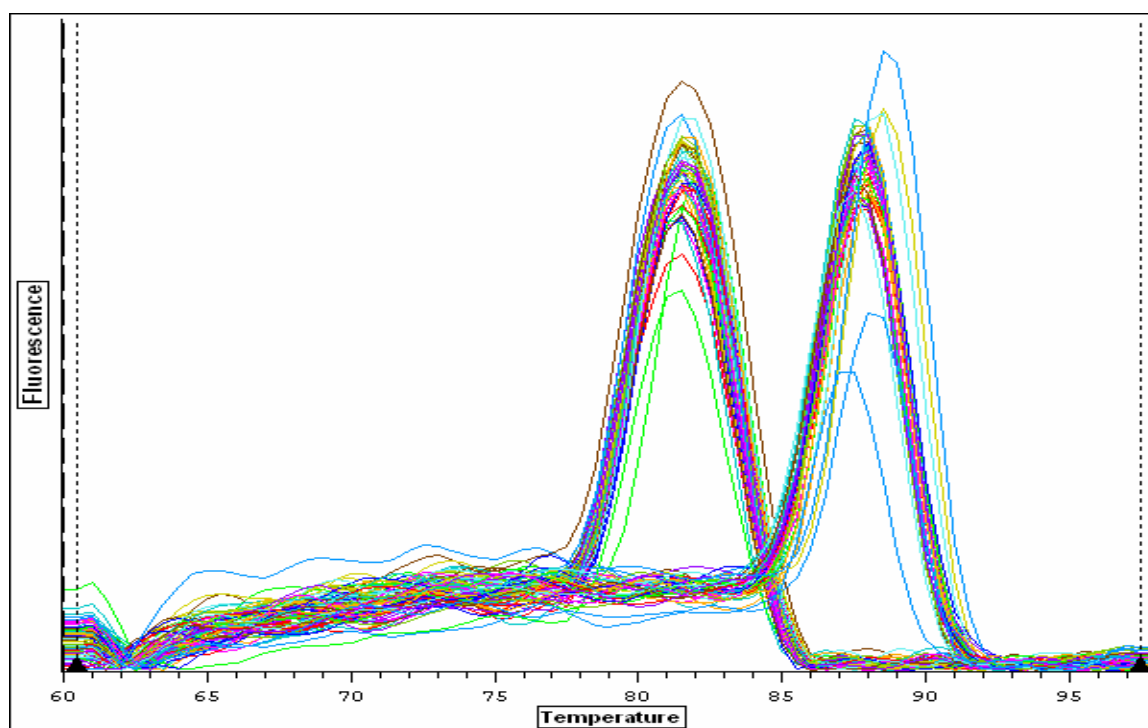
MELTING CURVE

Figure 2

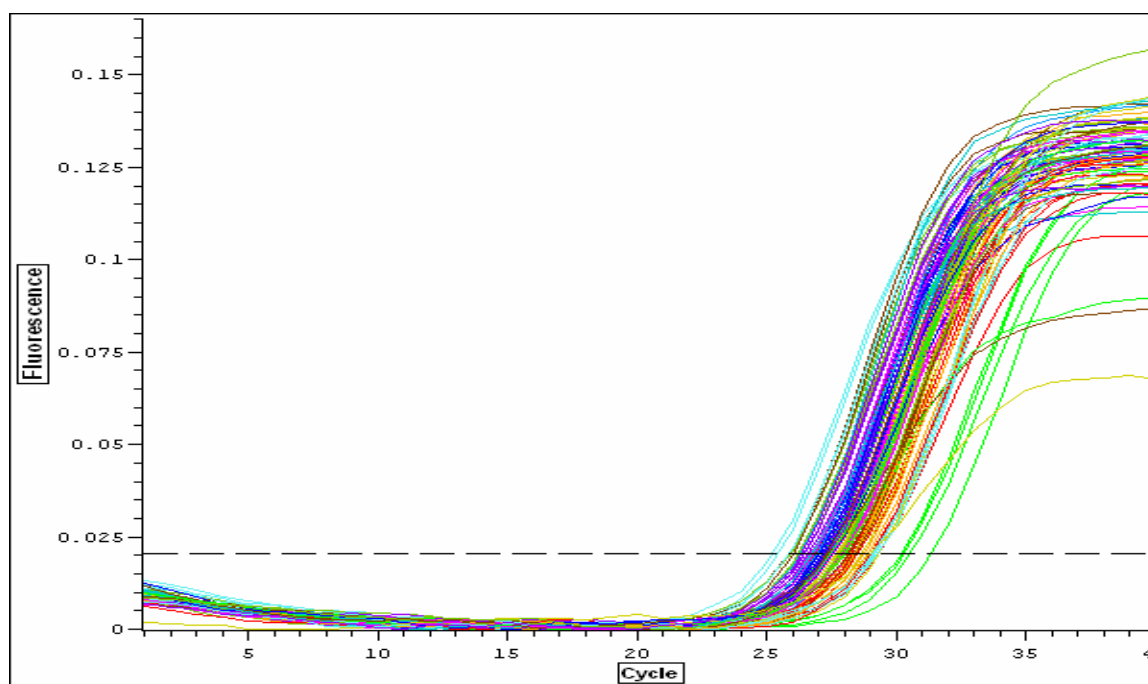
AMPLIFICATION CURVE

Figure 3

SAMPLE SIZE DETERMINATION:

As there was no Indian data regarding the frequency of defensin copy number polymorphisms in the general population, we calculated sample size assuming arbitrary frequencies of 5% for the general population and 25% for patients with ulcerative colitis. We calculated that a sample size for this study of 59 cases and 59 controls would detect the above difference with a power of 80% and an alpha value of 0.05. Patients with irritable bowel syndrome or functional bowel disorder were chosen as the control subjects because by definition these patients do not have a structural disorder of the bowel, and they are drawn from the same population as the cases.

STATISTICAL ANALYSIS:

For a statistical analysis, we used the SPSS (version 11). For each of the parameters that are included, percentages, relative risks and 95% confidence intervals were calculated between the cases and controls. Significance of the associations was calculated using the Chi Square test or Fisher's exact test and P value of less than 0.05 was considered to be of statistical significance.

RESULTS

Demographic characteristics:

Cases: 75 cases of chronic ulcerative colitis were included in this study with mean age being 37 years (range 15–70 years). 44 were male and 31 were female (Figure 4). They hailed from various states like Tamil Nadu, West Bengal, Jharkhand, Andhra Pradesh and others. Their body mass index mean (SD) was 20.7 (4.3).

Controls: 75 age, sex and religion matched controls were studied for the epidemiological associations and for study of defensin gene copy number polymorphism. The mean age of controls was 38 years (range 18-66 yrs) of which 43 were male and 32 were female (Figure 3). Their state of origin corresponded to the cases. Their body mass index was 21.3 (4.8).

Figure4

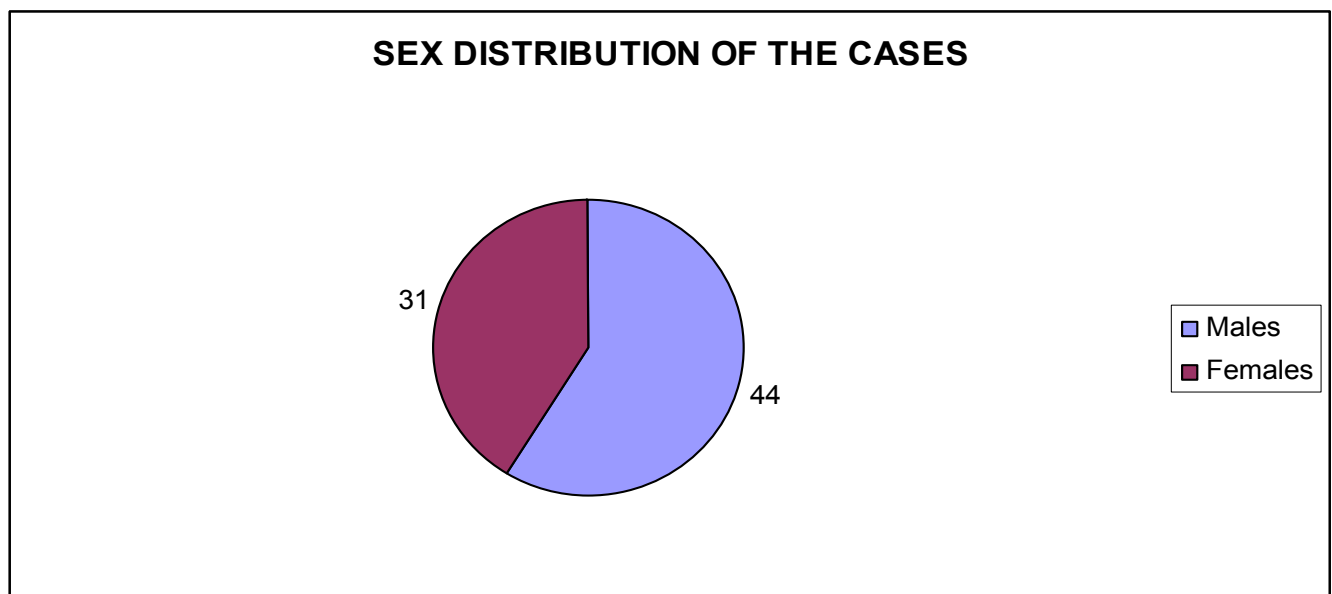
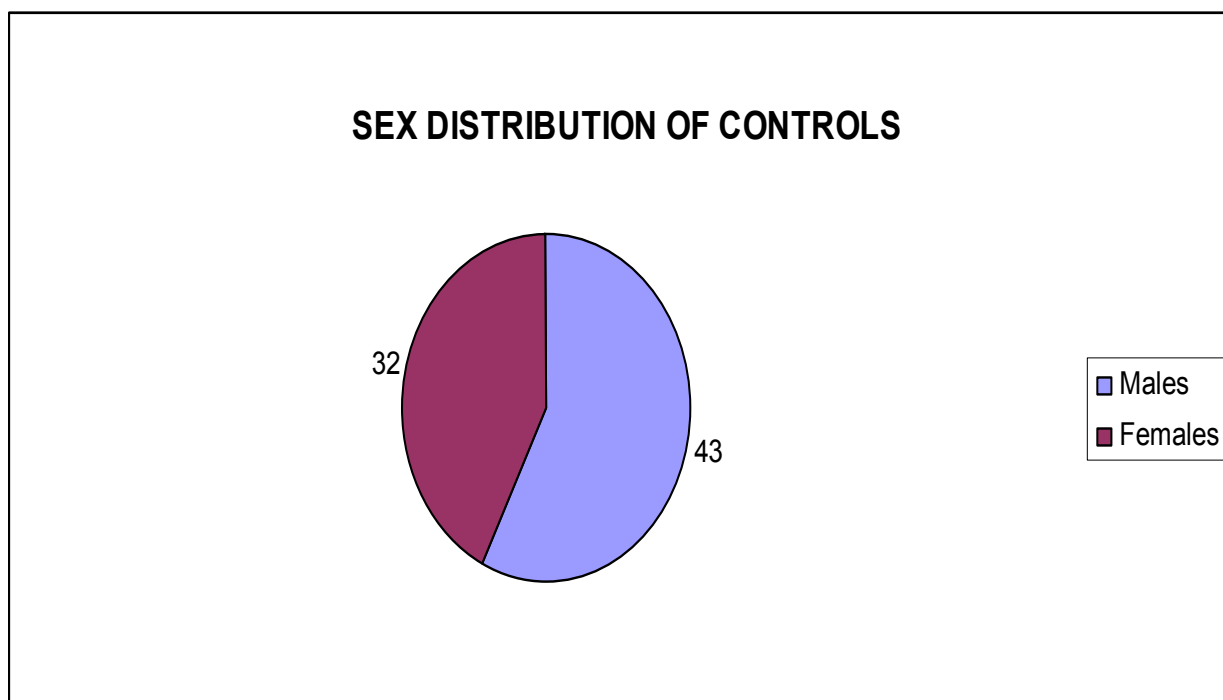


Figure5

Epidemiological associations and risk factors:

a) Place of residence, domestic water supply, and toilet availability. These are all factors that determine the level of domestic hygiene. The increasing incidence of inflammatory bowel disease with modernization has been attributed to the way in which the intestinal immune system is conditioned, especially in childhood, and this is the reason why these parameters were analyzed. The results of this analysis are shown in Table 2.

TABLE- 2

RISK FACTORS	ULCERATIVE COLITIS	CONTROLS	RR	95% CI	P
Childhood Residence Urban Village	31 44	30 45	1.05	0.56 – 2.02	1.00
Current Residence Urban Village	39 36	43 32	0.80	0.42-1.53	0.62
Childhood Water source Protected Unprotected	10 65	6 69	1.76	0.608 -5.144	0.42
Current water source Protected Unprotected	41 34	29 46	1.91	0.998 – 3.664	0.07
Childhood toilet Closed Open	44 31	36 39	1.53	0.806 – 2.932	0.252
Current toilet Closed Open	68 7	66 9	1.32	0.46 – 3.76	0.79

* Fisher exact test was used to compare these dichotomous variables, none of the differences was statistically significant.

In this study, we did not find any statistically significant association of birth place (village or town), place of current residence (town or village), having a closed toilet in the house, with the diagnosis of ulcerative colitis. The usage of protected water showed a trend towards significance ($p = 0.07$) indicating that there could be a possible association if we studied more cases and controls. The usage of protected water was associated with nearly 1.9 times (CI 0.99 – 3.67) increased risk of developing ulcerative colitis compared to controls.

b) Personal habits and diagnosis of ulcerative colitis:

TABLE 3

RISK FACTORS	ULCERATIVE COLITIS	CONTROLS	RR	95% CC	P
Toothpaste use in childhood Users Non users	38 37	31 44	1.45	0.76 – 2.78	0.32
Toothpaste usage in Current Users Non users	72 3	66 9	3.27	0.85 - 12.6	0.13
Worm infestation in childhood Present Absent	31 44	41 34	0.584	0.30 – 1.11	0.071
Exposure to Pets in childhood Yes No	27 48	21 54	1.45	0.72 – 2.89	0.38
Exposure to Pets now Yes No	14 61	9 66	1.68	0.68 – 4.17	0.37
Exposures to Cows / Poultry In childhood Yes No	39 36	38 37	1.05	0.56 – 2.001	1.00
Exposures to Cows / Poultry Now Yes No	17 58	13 62	1.39	0.62 – 3.13	0.54
Breast Fed Yes No	72 3	73 2	0.65	0.10 – 4.05	1.00
Separate Bedroom in childhood Yes No	12 63	3 72	4.571	1.234 – 16.935	0.02

In this study we also compared various other risk factors for development of ulcerative

colitis like usage of toothpaste in childhood, pets in the household, cattle and poultry in

the household, having a separate bedroom in childhood and breast feeding in infancy between cases and controls.

In this study we did not find any significant difference in any of these putative risk factors except that the availability of a separate bedroom for the person in childhood was significantly associated with ulcerative colitis, with a relative risk of 4 (CI 1.23 – 16.93) (P=0.02). A separate bedroom in childhood probably signifies higher socioeconomic status and may be a surrogate marker of higher hygiene status in childhood.

Socio Economic Status:

Socio economic status of the cases and controls was also calculated (current status) to assess whether it has any effect on incidence of ulcerative colitis and also to see if it is an indirect marker of sanitation and health. The modified Kuppuswamy scale has been in wide use since 2 decades, and this was used for calculation of social economic status of subjects in this study. This scale is a composite score of per capita monthly income, education of head of family and profession of the head. It gives maximum score of 29 and lowest score of 3. There was no significant difference in social or economic score.

Table- 4. Socio-economic Status:

	Cases	Controls	P-value
Social Score	17.2 \pm 5.2	17.1 \pm 4.4	0.8
Economic Score	5.6 \pm 2.2	5.7 \pm 1.9	0.7

Risk Factors Associated with Past Medical, Family and Personal

History:

In the past medical and surgical history, we collected the data regarding the history of appendectomy, diabetes mellitus, hypertension and other abdominal surgeries (Table 4).

In the family history we collected history of inflammatory bowel disease and consanguinity (Table 5). In the personal history we obtained history regarding smoking habits, alcohol consumption (Table 6) and types of food consumed, vegetarian or non vegetarian (beef, fish, pork, meat) (Table 7).

Table-5. Past Medical and Family History in Cases and Controls:

RISK FACTORS	ULCERATIVE COLITIS	CONTROLS	RR	95% CI	P
Appendectomy Done Not done	1 74	3 72	0.32	0.33 – 3.19	0.62
Had abdominal Surgery Yes No	10 65	16 59	0.56	0.23-1.34	0.28
Diabetes Mellitus Present Absent	5 70	7 68	0.694	0.21 – 2.29	0.76
Hypertension Present Absent	5 70	4 71	1.27	0.32 – 4.91	1.00
Family history of IBD Present Absent	2 73	0	2.027	1.72 – 2.38	0.248
Consanguinity Present Absent	8 67	8 67	1	0.36 – 2.82	1.00

Table-6. Personal Habits

	ULCERATIVE COLITIS	CONTROLS	RR	95% CI	P
Present					
Smokers	2	13	0.246	0.67 – 2.90	0.005
Non Smokers	73	62			
Past					
Smokers	11	23	0.58	0.35 – 0.97	0.03
Non smokers	64	52			
Tobacco Chewing					
Yes	12	13	0.9	0.38 - 2.14	1.00
No	63	62			
Alcohol					
Yes	12	14			
No	63	61	1.2	0.51 – 2.81	0.69

Table-7. Food Habits:

	ULCERATIVE COLITIS	CONTROLS	RR	95% CC	P
Vegetarian					
Yes	13	5	2.93	0.99 – 8.7	0.07
No	62	70			
Beef					
Yes	17	9	0.465	0.19 – 1.12	0.13
No	58	66			
Fish					
Yes	62	67	1.7	0.68 – 4.52	0.34
No	13	8			
Pork					
Yes	4	2	0.486	0.86 – 2.74	0.68
No	71	73			
Meat					
Yes	61	65	1.49	0.61 – 3.60	0.5
No	14	10			

A history of current cigarette smoking or past tobacco smoking were both protective against ulcerative colitis. Current smoking reduced the relative risk of ulcerative colitis to 0.246 (P=0.005); i.e. smokers were four times less likely to develop

ulcerative colitis. Past smokers had a relative risk of developing ulcerative colitis of 0.58 ($P=0.03$), indicating a protective effect of 42%. However, tobacco chewing did not show any significant association with ulcerative colitis. Previous studies have shown that alcohol consumption, western type of diet consumption especially like ham, meat were associated with increased incidence and increased relapse of ulcerative colitis. However, in this study, dietary factors did not appear to significantly influence the development of ulcerative colitis. In this study, we did not get any significant family history of IBD or history of consanguinity in the cases or controls.

CLINICAL FEATURES OF ULCERATIVE COLITIS

PATIENTS:

Table-8. Age of onset of the disease

Age range of Incidence of cases	Number of cases	Percentage
1 – 10	1	1.34
11 – 20	8	10.67
21 – 30	22	29.34
31 – 40	28	37.34
41 – 50	9	12
51 – 60	6	8
61 – 70	1	1.34

The above table shows frequency of onset of ulcerative colitis at various ages.

The above table shows that most of the cases were in middle age group that is mostly 3rd and 4rd decades ^(91, 92) with incidence rate of 50cases (66.68%). It is keeping in line with

other studies in which incidence was in the same decade as mentioned above but the second peak which was mentioned in the 6th decade was not seen in our study ⁽⁹³⁾.

Table-9. Symptoms of ulcerative colitis patients:

	Number of Cases	Percentage
Abdominal Pain	54	73.3
Diarrhoea	73	97.3
Blood in stool	73	97.3
Urgency	70	93.3
Tenesmus	59	78.7
Incontinence	44	58.7
Tiredness	64	85.3
Fever	21	28
Loss of weight	58	77.3
Arthritis	55	73.3

Most common symptom in ulcerative colitis was diarrhea with blood and mucus, and stools associated with urgency. These symptoms were present in 93 to 97% of the cases. Other symptoms like abdominal pain, tenesmus were little less around 70 to 75% and fecal incontinence was present in 58% of the cases. Arthritis was the only complication which was common extra intestinal manifestation in our study which was present around 73% of the cases. The high incidence of arthritis and of fecal incontinence could be secondary to referral bias.

Table-10. Extent of colitis:

Colon Extent	Number of Cases	Percentage
Pan colitis	44	58.7
Left sided colitis	17	22.7
Proctitis	14	18.7

Colonoscopically the ulcerative colitis was diagnosed by the presence of symmetrical and continuous inflammation that began in the rectum and extended proximally without interruption for entire extent of disease. Other features were decrease or loss of normal vascular pattern with erythema, edema of mucosa, and presence of granularity, friability with or without yellow brown mucopurulent exudates associated with mucosal ulceration and presence of pseudopolyps in long standing cases. When the above features suggestive of ulcerative colitis were present beyond the splenic flexure, it was considered as pan ulcerative colitis and if present till splenic flexure, considered as left sided colitis. If the above features were localized to the rectum it was considered as proctitis. In this study, pan ulcerative colitis was seen in 44 cases (58.7%), left sided colitis in 17 cases (22.7%) and proctitis in 14 cases (18.7%).

Table- 11. Clinical course of the disease in ulcerative colitis patients:

	Number of Cases	Percentage
Intermittent flare with complete resolution	42	56
Mostly continuous illness	23	30.7
Asymptomatic after initial attack	7	9.3
First Episode	3	4

Most of the cases included in this study were already on treatment instituted here or elsewhere. During the study, only three cases presented for the first time. As seen in Table 11, the majority of our patients, 56%, had intermittent flares of disease with complete remission between flares, which is also common presentation in other studies⁽⁹⁴⁾. Mostly continuous disease was seen in 30.7% cases. Quiescent disease after the first attack was seen in 9.3% cases.

Table 12. Grade of Ulcerative Colitis (Histology):

Grade	Number of Cases	Percentage
Mild	19	25.34
Moderate	43	57.34
Severe	12	16.00
Quiescent	1	1.3

Table 13. Laboratory Parameters

	Mean \pm SD
Hemoglobin g%	10.9 \pm 2.3
ESR mm/h	41.8 \pm 28.19
Albumin g%	3.76 \pm 0.71

Most of our patients were on treatment by the time of presentation. So, Hb, albumin and ESR were within the normal range. Hb. is being 10.9 \pm 2.3gm%, albumin 3.76 \pm 0.7 and ESR 41.8 \pm 28.1.

The Ulcerative Colitis Disease Activity Index (UCDAI) is the sum of scores of 4 components, stool frequency, rectal bleeding, sigmoidoscopic findings and physician's global assessment. This disease activity index ranges from 0 to 12 with a higher total score representing more severe disease. Disease activity index in our study was 7.11 \pm 3.67 which indicates most of these patients had mild or moderate activity, most probably secondary to the treatment.

The Seo's disease activity index⁽⁹⁴⁾ was also used for quantification of the disease activity. The activity index (AI) is calculated using the formula: AI = 60 x bloody stool + 13 x bowel movements + 0.5 x ESR – 4 x Hb – 15 x Albumin + 200 and the above values are graded as less than 150 as mild, 150 – 220 as moderate and greater than 220 as severe.

Table-14. Seo's Activity Index

Mild (< 150)	31	41.34
Moderate (150 – 220)	27	36.0
Severe (> 220)	17	22.67

In this study, around 40% patients had mild disease and 36% patients had moderate disease and around 22% patients had severe disease as per the Seo's activity index which was in correlation with the ulcerative colitis activity index.

Table -15. Treatment

DRUG HISTORY	NUMBER OF PATIENTS	PERCENTAGE
H/O PAST AMINO SALICYLATE CONSUMPTION		
YES	55	73.3
NO	20	26.7
PAST ORAL STEROID CONSUMPTION		
YES	41	54.7
NO	34	45.3
PAST RECTAL STEROID USAGE		
YES	20	26.7
NO	55	73.3
PAST H/O IV STEROIDS		
YES	9	12
NO	66	88
PAST H/O IMMUNOSUPPRESSION		
YES	16	21.3
NO	59	78.6
PRESENTLY ON SALICYLATES		
YES	50	66.7
NO	25	33.3
PRESENTLY ON STREOIDS		
YES	21	28
NO	54	72
PRESENTLY ON IMMUNOSUPPRESSION		
YES	11	14.7
NO	64	85.3

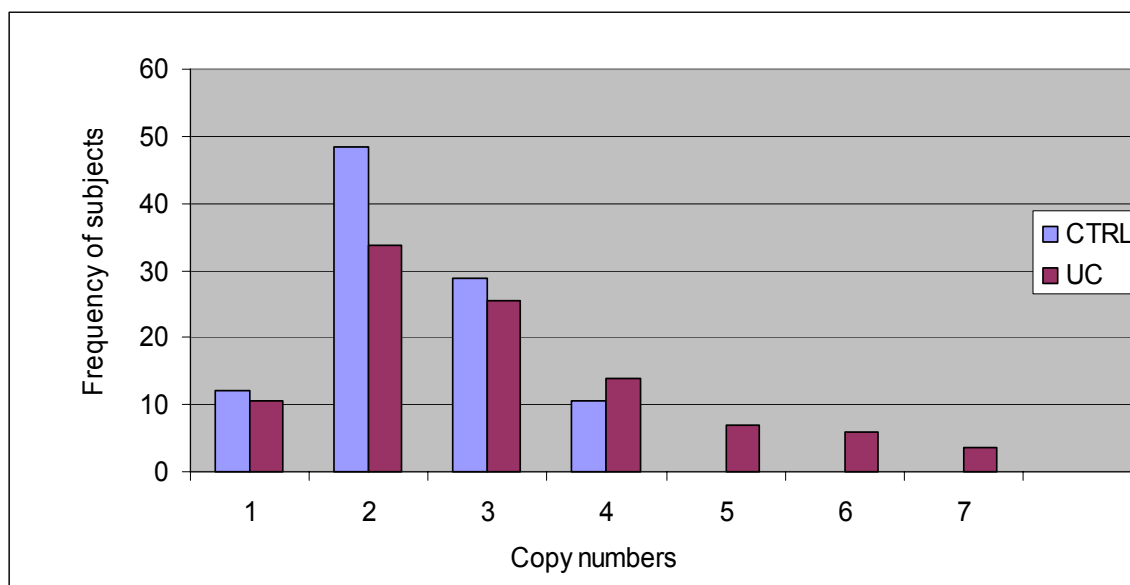
Most of the patients mostly on salicylates treatment is around 66.7% only few of them work on steroids 28% only few needed immunosuppression that is around 14.7%.

HUMAN BETA-DEFENSIN 2 COPY NUMBER

POLYMORPHISMS

The human beta-defensin gene 2 (also known as DEFB4) copy number polymorphism results are shown in the Figure 6. The majority of the subjects had less than 4 copies of DEFB4 gene but significant number of the cases had 4 or more copies when compared to controls which were statistically significant ($p=0.005$). Subjects with defensin gene copy numbers ≥ 4 had relative risk of 2.85 times to develop UC. Thus the presence of four or more copies of this gene may be one of the causes of the pathogenesis of the ulcerative colitis.

Figure 6



$P=0.005$ for 4 or more gene copies UC Vs controls. Fisher exact test.

TABLE 16

COPY NUMBER POLYMORPHISMS	CASES	CONTROLS	P value*	RR OF CNP
<4	60	59	0.005	2.85(1.32– 6.16)
≥4	26	7		

*Fisher exact test

DISCUSSION

Ulcerative colitis is a disease which was described mostly in the developed countries. There are only a few studies from developing countries like India regarding incidence of ulcerative colitis which was increasing in present days and to see the effect of hygiene on the incidence and course of IBD. A study from the Punjab showed that prevalence of UC was nearly similar in India and in Western countries⁽¹¹⁵⁾.

The hygiene hypothesis has been developed to indicate why certain immune diseases are increasing in the modern world. If ulcerative colitis is increasing in India, perhaps it could be due to recent changes in the social practices, diet patterns and hygiene in India. Therefore this was undertaken as a case-control study to examine the effect of these parameters on the diagnosis of ulcerative colitis.

The incidence of the ulcerative colitis in our population was approximately in the same age group as described by Yang SK et al⁽⁹³⁾ and Probert C .S et al⁽⁹⁴⁾ described in 2000 and 1996 respectively, that incidence was common in the 2nd and 3rd decades. As our study was done at a point of when patients presented to our institution but were not followed the 2nd peak as described by Yang SK et al was not observed. Equal sex incidence in our study was almost keeping with the two other studies described above.

The body mass index of this study group was within the range as described by Kuganthosan.S. et al⁽⁹⁷⁾ and Jabpsen et al⁽⁹⁸⁾.

Various studies examined factors like geographical factors, environmental factors, social status, and microbial agents in the pathogenesis of IBD. Data supporting the involvement of each of these factors in predisposing to, triggering (or) modulating the cause (or) outcome of IBD vary from strong to tenuous. Data by Silvio Danere et al⁽¹⁰⁰⁾,

AE. Gent et al ⁽⁹⁹⁾ suggested that there was no effect of the environmental factors and childhood hygiene as the outcome of ulcerative colitis except that study by Gent et al found that usage of protected water in childhood was associated with increased incidence of the ulcerative colitis. This may be due to immune conditioning of the gastrointestinal tract.

In this study, we also found that there was no significant difference between the cases and controls in factors like childhood environmental conditions such as residence in village (or) urban area, usage of closed toilet (or) history of worm infestation. Like the study by Gent et al who found that hot water taps were more common in houses of subjects with IBD, in this study also people who consumed protected water had a relative risk of 1.96 when compared to people not consuming the protected water and increased risk may be secondary to the immune condition of the GI tract by the GI infections.

Parasitic helminths are powerful modulators of their host's immune system. It is suggested that the reduced exposure to helminths, due to better hygiene condition, may predispose to the development of IBD. Helminths can activate Th2-type immune responses and down regulate Th1-type response. The latter are responsible for production of interferon-gamma-tumor necrosis factor-alpha release and promote interleukin-10 transforming growth factor-beta and regulatory T-cell production. Thus helminths can prevent and reverse intestinal inflammation.⁽¹⁰¹⁾ The study by Gyaene et al ⁽¹⁰²⁾ has showed that helminth infestation was protective. In our study we found that worm infestation in childhood was reaching significance (P=0.07) but not completely significant as the history was taken depending on the ability of the patients to recall this.

Around 10 cases (13%) were not sure of the worm infestation in childhood, and these were considered as not having the infection.

Tooth paste which contains micro particles could also serve as proxy measures of hygiene conditions (or) have an impact on the microbial flora of the gut. However observational studies did not show any association implicating tooth paste as an independent risk factor which was shown in our study also which was consistent with the study by Bussel M.G. Et al in 1998 ⁽¹⁰³⁾.

In our study we studied the relation of pets (or) exposure to partly which did not show any significance which was the same in other studies by Benotein et al in 2006 ⁽¹⁰⁴⁾.

Breast feeding appears to be protective and its effect on ulcerative colitis was evidenced by various studies and a meta-analysis was done by Klement E et al⁽¹⁰⁵⁾ in 2004 which revealed odds ratio ranged from 0.56 to 0.77 depending on the quality of study. Even in our study the odds ratio was 0.65 indicating that breast feeding was possibly associated with protection from the ulcerative colitis.

Having a separate bedroom in childhood is the one factor in our study highly significant with a p value of 0.02 and relative risk of 4.57 (1.23-16.03) indicating that having a separate room from childhood indicating higher socio economic studies and also may reflect various psychosocial effects.

There was no difference in the socio economic status of the cases and controls. This difference was noted as against the other studies may be because our subjects presented to the tertiary hospital rather than being recruited from the community. Therefore referral bias could have affected the socio economic status of these individuals.

Human appendix has been considered as a vestigial remnant, many case control studies suggest that previous appendectomy is rare in ulcerative colitis. In the appendix of the ulcerative colitis patients, the CD4/CD8 ratio is significantly increased and the proportion of CD4 + CD69 + (early activation antigen) T cells, but not of CD4+HLA-DR4 (mature activation antigen). T Cells is also significantly increased. These findings suggest that the appendix may be primary site in development of ulcerative colitis ⁽¹⁰⁶⁾. In our study we found that OR of 0.32 for not developing the ulcerative colitis (CI 0.3-3.19) but this was not statistically significant. It was a little high when compared to study by Desanssure petat ⁽¹⁰⁷⁾ who found OR of 0.1 (95% CI 0.05-0.21) ⁽¹⁰⁷⁾. We did not find any much difference in the incidence of ulcerative colitis in patient has had previous abdomen surgeries between the cases and controls.

We found that family history of IBD in the first degree relative had an increased risk by 2, seen in 2 patients (2.06 of patients). There are consistent with the study done by park JB et al in 2006 who studied in Korean population found to have incidence of ulcerative colitis was around (2.01%) ⁽¹⁰⁸⁾.

The relation between smoking and IBD is complex ulcerative colitis is largely a disease of non-smokers and former smokers Cigarette smoking may even result in a beneficial influence on the course of ulcerative colitis. The potential mechanisms involved in this dual relationship include changes in hormonal and cellular immunity, cytokine and eicosanoid levels, gut motility, permeability, blood flow, colonic mucosa and oxygen free radicals. Nicotine is assumed to be the active moiety ⁽¹¹⁰⁾. A meta analysis of Mahid SS et al ⁽¹⁰⁹⁾ found that current smoking had a protective effect on

development of ulcerative colitis when compared with controls with OR-0.58 (95%CI 0.45-0.75).

In one study we found that relative risk was increased 7 times (CI 1.67-35.2; P-0.005) in nonsmoker when compared to present smokers. We also found that past smokers were protected 2.58 times (95 % CI 1.14-5.76) when compared to non-smokers with P value of 0.03. But we did not find any protective effect of tobacco chewing (or) alcohol consumption.

Various studies were done in different geographic areas with various types of dietary habits and the results varied. In our study there was significant association between the various types of food consumed with OR ranging from 0.486 for having pork to 2.93 being a vegetarian. It appears that strict vegetarians were at a risk of 2.93 compared to non-vegetarians although this did not reach statistical significance. Zuinbliene et al ⁽¹¹¹⁾ also studied various dietary habits but were not able to establish any specific association with any food habits.

Tables 17 and 18 show differences between the present study and one by Park et al ⁽¹¹²⁾. The difference in the extent of colitis, severity, between the park SH et al and our study may be because most of the patient who were included in there study were first diagnosed cases but in our study were mostly referred cases which did not respond to routine treatment or who had relapse so in our study we had patients mostly with pancolitis and mild to moderate in severity because of treatment.

Table 17

Clinical features	Park SH et al	Out Study
M : F	0.94 : 1	1.59 : 1
Median Age	40 (12 – 72 yrs)	38 (15 – 20 yrs)
Proctitis	134 pts (44.1%)	14 pts (18.7%)
Left sided colitis	69 pts (22.7%)	17 pts (22.7%)
Pan colitis	101 pts (33.2%)	44 pts (58.6%)
Mild	149 pts (49%)	19 pts (25.34%)
Moderate	125 pts (41.1%)	43 (57.34%)
Severe	26 pts (86%)	12 (16%)
Quiscent	--	1 (1.3%)

Table 18

Clinical Course	Park SH et al	Cases (percentage)
First episode	100 %	3 (4%)
Asymptomatic after initial attack	97.4%	7 (9.3%)
Mostly continuous illness	-	23 (30%)
Complete resolution		42 (56%)

Defensins are antimicrobial peptides produced by professional phagocytes, Paneth cells, and intestinal epithelial cells. Current data favor the hypothesis that defensins and

Paneth cells⁽¹¹⁴⁾ may play important roles in the maintenance of intestinal immune homeostasis.⁽¹¹⁶⁾ Rahaman et al⁽¹²⁰⁾ demonstrated that lamina propria in colon from UC patients, Crohn's colitis patients, and controls contain cells that express hBD-2. They proposed that plasma cells were 2-3 times more abundant in UC colon than in control colon and Crohn's colitis. Moreover, plasma cells in UC colon expressed hBD-3 and hBD-4 mRNA. Additionally, hBD-2 mRNA expression was demonstrated in 3 out of 4 well-characterized plasma cell lines and they concluded that mature colonic plasma cells can express multiple β -defensins. In UC, defensin production by plasma cells is probably clinically relevant since plasma cells accumulate in large numbers between the distorted crypts and muscularis mucosa.⁽¹¹⁷⁾ A study by Fellermann⁽¹¹⁹⁾ who tested the genome wide DNA copy number profiling by array-based comparative genomic hybridization and quantitative polymerase-chain-reaction analysis of the human β -defensin 2 (HBD-2) gene they that healthy individuals, as well as patients with ulcerative colitis, have a median of 4 (range 2-10) HBD-2 gene copies per genome. In a surgical cohort with ileal or colonic CD and in a second large cohort with inflammatory bowel diseases, those with ileal resections/disease exhibited a normal median HBD-2 copy number of 4, whereas those with colonic CD had a median of only 3 copies per genome ($P=.008$ for the surgical cohort; $P=.032$ for the second cohort). Individuals with ≤ 3 copies have a significantly higher risk of developing colonic CD than did individuals with ≥ 4 copies (odds ratio 3.06; 95% confidence interval 1.46-6.45) and they concluded that a lower HBD-2 gene copy number in the β -defensin locus predisposes to colonic CD, most likely through diminished β -defensin expression.⁽¹¹⁸⁾ In our study we tested the copy number polymorphism of the β -defensin gene-2 which showed that patients with

ulcerative colitis had copy numbers 4 and more compared to controls (RR – 2.85 , CI – 1.32 -6.16 , p -0.005), this was consistent with the study by Fellerman. Probably the higher expression may be due to higher concentration of the plasma cells in the ulcerative colitis patients compared to controls as explained by Rahmann et al. In conclusion it indicates that DEFB-4 gene copy number is more in UC patients compared to healthy people. Since our study population was in low number may need large number to definitely conclude the excess expression of this gene polymorphism in ulcerative colitis.

CONCLUSIONS

This case control study compared cases with ulcerative colitis and controls with irritable bowel syndrome and came up with the following findings:

1. UC patients had mean age of onset of disease in third and fourth decades with no sex predilection and well preserved body mass index.
2. Among several risk factors concerned with domestic hygiene only usage of protected water, lack of parasitic infestation in childhood and having a separate bedroom appeared to have significant risk for development of the ulcerative colitis.
3. Among the personal habits, present smokers and past smokers were protected from development of ulcerative colitis.
4. There was no association of type of diet consumed and diagnosis of UC.
5. Defensin gene DEFB-4 copy number polymorphism testing revealed that copy number polymorphism of 4 and ≥ 4 were associated with ulcerative colitis
6. Apart from the blood mixed stools, urgency was the common presentation.
7. Arthritis was the most common extra intestinal manifestation of ulcerative colitis
8. Most of the patients had intermittent flares with complete resolution between flares.
9. Pancolitis with moderate severity was the common presentation
10. Disease was well controlled by medications like salicylates and oral steroids as most of the patients had mild activity with well preserved hemoglobin and albumin.

SUMMARY

This case control study of patients with ulcerative colitis seen in a tertiary referral hospital established that patients had onset of disease mostly in third and fourth decade of life, commonly had intermittent flares of disease, and usually had pancolitis and disease of moderate severity. Factors that were associated with a diagnosis of ulcerative colitis included consumption of protected water in childhood, having a separate bed room in childhood and absence of intestinal parasite infection in childhood. Socio economic status, type of food consumed and birth or residence in a village were not associated with the disease. Smoking, either current or past, was generally protective. Of interest, copy numbers of the human beta-defensin 2 (DEFB4) gene were higher in cases of ulcerative colitis (≥ 4) compared to controls. This could be important in the pathogenesis of ulcerative colitis.

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PROFORMA FOR ULCERATIVE COLITIS

Patients name		Hospital No.	
Contact Address			
Age in years	Sex	Male / Female	State of
Origin			
Weight (Kg.)	Height (Cm)	Body Mass	
Index			

Epidemoligical information:

At present where do you live?	Urban (1) / Village (2)
When you were born, where did your mother live?	Urban (1) / Village (2)
Do you remember passing worms in your stool as a child?	Yes (1) / No (2)
Did you use filtered or boiled water for drinking as a child? .	Yes (1) / No (2)
Do you use filtered or boiled water for drinking currently?	Yes (1) / No (2)
Did you have tap water in your home as a child?	Yes (1) / No (2)
Do you currently have running tap water in your house?	Yes (1) / No (2)
Did you ever have a home with a closed toilet system when you were a child?	Yes (1) / No (2)
Do you currently have a closed toilet system at home?	Yes (1) / No (2)
Usage of tooth paste for cleaning teeth as a child	Yes (1) / No (2)
What do you use for cleaning teeth now? Toothpaste	Yes (1) / No (2)
Did you sleep alone in a separate room as a child?	Yes (1) / No (2)
Did you keep pets at home <u>as a child?</u>	Yes (1) / No (2)
Do you keep pets at home now?	Yes (1) / No (2)
Were there cows, buffaloes, goats, or poultry in your house or yard as a child?	Yes (1) / No (2)
Were there cows, buffaloes, goats, or poultry in your house or workplace now?	Yes (1) / No (2)
Were you breast fed?	Yes (1) / No (2)

Socio economic status: (modified Kuppuswamy scale)

- A. **Social Score:-**
a. House b. No house c. Katcha House d. Mixed House
e. Pucca House f. Mansion

B. **Material possessions (tick all applicable)**

Score:	Criteria	Article No.	Final score
0.	None	1 Cycle	1. Radio
	Furniture more than 3 pieces		
	Electrical fittings;		Bullock Cart
	Sewing Machine		Well
	Motor cycle/moped/scooter		Improved agricultural implements
	TV/Tape/VCR		Vessels in brass More than 5

C. **Education (mark highest score in household)**

Illiterate	0	Can read only	Can read and write
Primary (Grade 1-5)		Middle School	(grade 6-8)
High School (grade 6-8)		High Schoo	(grade 9-10)
Higher Secondary (11-12)		Above 12 th grade	.
Total social score (A+B+C)			
.			

Economic Score:

A) Occupation: mark only highest score for household:-

Labour (Agri/unskilled labourer)	Labour (skilled)
Small business and trader	Independent professions
Cultivator owner/tenant/landowner	Service (Govt./Private/Ex Service

b) No of persons employed:

Total Economic score = (A+B)

Patient History:-

At what age did symptoms appear?

Have you ever had the following symptoms in relation to your illness?

Abdominal pain	Yes (1) / No (2)
Diarrhoea	Yes (1) / No (2)
Blood in stool	Yes (1) / No (2)

Past Medical History

Diabetis Mellitus	Yes (1) / No (2)
Hypertension	Yes (1) / No (2)

Past Surgical history

Have you had appendicectomy	Yes (1) / No (2)
History of any abdominal surgery	Yes (1) / No (2)

Family History:

Has any member of your family been ever diagnosed to have? IBD /Chronic diarrhea

Personal habits:

Do you current smoke cigarettes / beedis?	Yes (2) / No (1)
In the past id you smoke cigarettes/ beedis?	Yes (2) / No (1)
Do you chew tobacco alone or with pan?	Yes (1) / No (2)
Do you take alcoholic drinks?	Yes (2) /No (1)

Dietary History:

Are you a strict vegetarian	Yes (1) / No (2)
Do you consume beef	Yes (2) / No (1)
Do you consume fish	Yes (2) / No (1)
Do you consume pork	Yes (2) / No (1)
Do you consume meat	Yes (2) / No (1)

Complications:

Have you ever had painful joint swellings	Yes (1) / No (2)
Have you had chronic low back pain	Yes (1) / No (2)

Medical treatment

Have you ever used aminosalicylates in the past?	Yes (1) / No (2)
Have you ever used oral steroids in the past	Yes (1) / No (2)
Have you ever used rectal steroids in the past	Yes (1) / No (2)
Have you ever used IV steroids in the past	Yes (1) / No (2)
Have you ever used azathioprine/other immuno Suppressants in the past	Yes (1) / No (2)
Are you currently using aminosalicylates	Yes (1) / No (2)
Are you currently using steroids (any form)	Yes (1) / No (2)
Are you currently using azathioprine/ Immuno-suppressants	Yes (1) / No (2)

Chinical course : 1.Intermittent flares with complete resolution in between
2. Mostly continuous illness
3. Asymptomatic after initial attack
4. First episode

Disease extent: 1. Pancolitis 2. Extensive colitis 3. Left sided colitis
4.Proctitis

Colonoscopy report

Biopsy report

Barium meal or CT report

Laboratory findings

Test	date	report
Hb (g/Dl)		
ESR (mm/h)		
S. Albumin		
Blood for genetic Studies		

Clinical activity index

Symptom	Score	Symptom	Score
Diarrhea (no. of daily stools		Nocturnal diarrhea	
0-2		No	0
3-6		Yes	1
7-9		Fecal incontinence	
10 or more		No	0
Visible blood in stool % of movement:		Add pain/cramping	
0	0	None	0
Less than 50	1		

More than 50	2	Mild	1
100	3	Moderate	2

General wellbeing

Perfect	0	Abdominal tenderness.	
Very good	1	None	0
Good	2	Mild/localized	1
Average	3	Mild to mod/diffuse	2
Poor	4	Severe or rebound	3

Need for antidiarrheal drugs

Total score

No	0
Yes	1

See Disease Activity Index for UC

<u>Symptom</u>	<u>Score</u>	<u>Weighting</u>	<u>Final score</u>
Bloody stool		x 60	
Little or none	0		
Present	1		
Bowel movements per day			x 13
≤ 4	1		
5-8	2		
≥ 8	3		
ESR (mm/h)			x 0.5
Hemoglobin (g/dl)			x -4
Albumin (g/dl)			x-15
Constant			200
Total score			

Consent Form

Dr. E.Umakanth ; Department of Gastrointestinal sciences, CMC. Vellore is currently carrying out genetic analysis of patients suffering from Ulcerative colitis or IBS persons as controls.

This document certifies that I _____ have voluntarily accepted to participate in this study without any force from the investigators and am willing to give 10 ml of my blood for the same. I have been assured that the information generated from these tests is purely for scientific research and the results will be kept confidential.

Signature of Participant

Signature of Doctor

Name of Participant

Date